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Short communication

Insulin-like growth factor II receptors in human brain and their absence in astrogliotic plaques in multiple sclerosis

Nadine Wilczak[a,*], Pieter De Bleser[b], Paul Luiten[c], Albert Geerts[c], Albert Teelken[a], Jacques De Keyser[a]

[a]*Department of Neurology, Academisch Ziekenhuis Groningen, PO Box 30.001, 9700 RB Groningen, The Netherlands*
[b]*Laboratory for Cell Biology and Histology, Vrije Universiteit Brussel, Brussels, Belgium*
[c]*Department of Animal Physiology, Rijksuniversiteit Groningen, Haren, The Netherlands*

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Abstract

Insulin-like growth factor (IGF) II receptors were studied in human adult brain by using autoradiography with [^{125}I]IGF-II. Receptors were found to be widely distributed throughout all neuronal regions. The highest densities were found in plexus choroideus, granular layer of the cerebellar cortex, gyrus dendatus and pyramidal layer of the hippocampus, striatum, and cerebral cortex. White matter was devoid of IGF-II receptors. We also examined [^{125}I]IGF-II binding in six plaques of multiple sclerosis, which were characterized by a dense network of astrocytes. We were unable to detect IGF-II receptors in any of the astrogliotic plaques, suggesting that IGF-II receptors in human brain are not involved in astrogliosis. The regional variations in neuronal distribution of IGF-II receptors suggest involvement of IGF-II in functions associated with specific neuronal pathways.

Themes: Development and egeneration

Topics: Neurotrophic factors: receptors and cellular mechanism

Keywords: Insulin-like growth factor II; Insulin-like growth factor II receptors; Human brain; Multiple sclerosis; Astrocytes

The insulin-like growth factors (IGFs), IGF-I and IGF-II, are mitogenic polypeptides with structural homology to insulin. Two major types of receptors recognize the IGFs and mediate their physiological effects: IGF-I and IGF-II receptors [6,8,9]. The IGF-I receptor is a heterotetrameric glycoprotein composed of two α and two β subunits linked by disulfide bounds [6]. The IGF-II receptor is a monomeric receptor with a striking extracellular domain made up almost exclusively of 15 cysteine-based repeats, and is identical to the cation-independent mannose-6-phosphate receptor [14,18]. In fact, these receptors display two distinctive binding sites: one for IGF-II and another for mannose-6-phosphate containing glycoproteins, which are mainly lysosomal enzymes that are endocytosed into endosomes. IGF-I receptors have been studied extensively in the central nervous system in both rodents [3,11,16] and humans [1,7]. The distribution of IGF-II receptors has been investigated in rodent brain [11,16,20]. However, there is only one report dealing with the presence of IGF-II binding sites in brain homogenates from a single individual [19]. Here we present the first autoradiographic study in human brain.

Brain tissue was obtained at autopsy from six patients (three females and three males), aged 65–78 years, who had died without evidence of neurologic or psychiatric disease. Slices of hemispheric white matter containing chronic plaques with dense astrogliosis, identified by glial fibrillary acidic protein (GFAP) immunoperoxidase staining, were obtained from four patients with multiple sclerosis (three females and one male; aged 59–78 years). Post-mortem delay ranged between 4 and 12 h. Tissue blocks of 0.5-cm thick were frozen rapidly by immersion in isopenthane–dry-ice or liquid nitrogen, and stored at −80°C until further processing.

Frozen tissue blocks were mounted on a cryostat chuck

*Corresponding author. Tel.: +31-50-361-2430; fax: +31-50-361-1707.

E-mail address: n.wilczak@neuro.azg.nl (N. Wilczak)

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coated with embedding medium and sections of 15-μm thickness were cut at −20°C using a microtome-cryostat, mounted on gelatin-coated glass slides.

Sections were preincubated for 15 min at 20°C in 25 mM Tris–HCl (pH 7.4), containing 10 mM $MgCl_2$, and 0.1% bovine serum albumin. Incubation experiments with [^{125}I]IGF-II (specific activity 2000 Ci/mmol; Amersham, UK) were done in duplicate at 20°C in the same buffer composition. For saturation and competition binding studies the sections were incubated for 120 min with [^{125}I]IGF-II. For saturation binding experiments nine concentrations of [^{125}I]IGF-II between 0.01 and 2 nM were used. Competition binding experiments with IGF-I and IGF-II were done with a concentration of 0.1 nM [^{125}I]IGF-II. After incubation, the sections were washed in ice-cold buffer to remove unbound ligand, wiped from the slides with Whatman GF/B glass fiber filters, and radioactivity was determined in a gamma-counter. Binding isotherms were analyzed by non-linear least-square curve fitting. K_D values were calculated from analysis of the Scatchard plots. The dissociation constants (K_i values) of IGI-I and IGF-II (Gropep, Australia) were calculated from the corresponding IC_{50} values according to the equation of Cheng and Prusoff [4]. Non-specific binding was obtained in the presence of 0.5 μM IGF-II. Specific binding was obtained by subtracting non-specific binding from the total binding.

For autoradiography, the sections were dried under a stream of cold air, placed in X-ray cassettes together with [^{125}I]standards (Amersham), and apposed to [^{3}H]Ultrofilm (Amersham) for 4 days and analyzed as described previously [7]. The regions of interest were sampled and mean optical densities determined, and converted into fmol/mg protein, based on the experimentally determined relation between polymer and brain paste standards (kindly provided by Amersham, UK).

For immunocytochemistry sections were fixed in 3% paraformaldehyde in 0.01 M phosphate buffered saline (PBS) for 15 min at room temperature and three times washed with PBS for 5 min. After blocking of non-specific background staining with 5% normal goat serum, the avidin–biotin–peroxidase complex was used. Sections were incubated in primary antibody solution (1/200) goat-anti-insulin-like growth factor binding proteins (IGFBPs) 1, 2, 3, 5 and 6 (Gropep) in PBS, overnight at 4°C. The secondary antibody was biotinylated, sections were incubated for 90 min at room temperature in rabbit-anti-goat (1/200) solution. Finally the sections were incubated in horseradish peroxidase-conjugated streptavidin in PBS for 90 min at room temperature and processed by the diaminobenzidine (DAB)/H_2O_2 reaction. Between all steps the sections were rinsed thoroughly with PBS.

The binding of [^{125}I]IGF-II to human cerebellum was saturable. Scatchard analysis of the saturation binding data revealed a K_D value (mean±S.E.M., n=3) of 0.79±0.06 nM (not shown). Competition curves with IGF-I and IGF-II were best fitted to a one-site binding model (Fig. 1). The calculated K_i value (mean±S.E.M., n=3) was 2.4±0.3 nM for IGF-II and 25.6±2.1 nM for IGF-I. The brain also contains IGFBPs, of which at least six types have been identified. These proteins can bind both IGF-I and IGF-II, and regulate their access to the IGF receptors [2,5,6,9]. By using immunocytochemistry on unwashed slices of frontal cortex we were able to identify IGFBPs 1, 2, 3, 5 and 6 on neuronal cell bodies. However, after preincubating the slices for 15 min at 20°C in the same buffer used by autoradiographic experiments, IGFBPs were no longer detectable in the slices (Fig. 2). Thus, IGFBPs were removed from the tissue slices during the preincubation procedure, indicating that in the experimental conditions that we used [^{125}I]IGF-II binding occurred to IGF-II receptors.

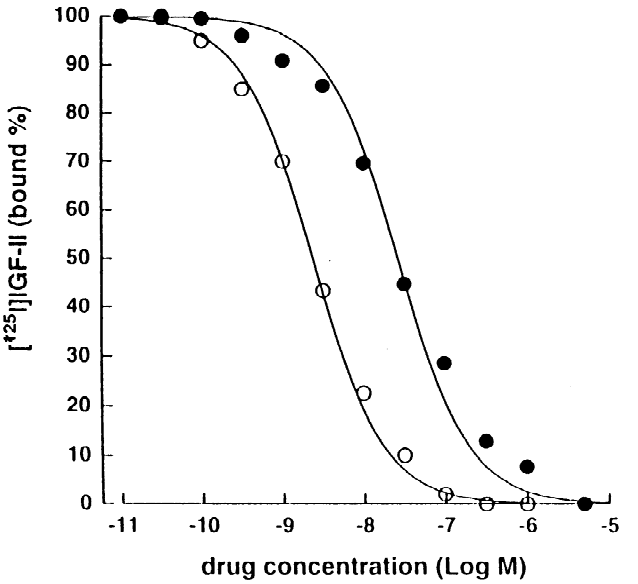

Fig. 1. Representative competition curves for IGF-II (○) and IGF-I (●) inhibition of 0.1 nM [^{125}I]IGF-II specific binding on microtome sections of cerebellum. Computer analysis of the binding data revealed that the competition curves were best described by a one-component binding model.

Similar to rodent brain [11,16,17,20], IGF-II receptors were found to be widely expressed throughout the neuronal regions of the human brain. The regional distribution of the IGF-II receptors is shown in Table 1. Representative autoradiographs are shown in Figs. 3 and 4. The highest density was observed in choroid plexus. Relatively high levels of IGF-II receptors were found in the different layers of the cerebral cortex and in the neostriatum (caudate nucleus, putamen, and accumbens nucleus). In the hippocampal formation, IGF-II receptors were concentrated in the granular layer of the dentate gyrus and the pyramidal layers of the CA1–CA3 subfields. In the cerebellar cortex, highest labeling was observed in the granular layer and low levels were seen in the molecular layer. In the midbrain, IGF-II receptors were mainly

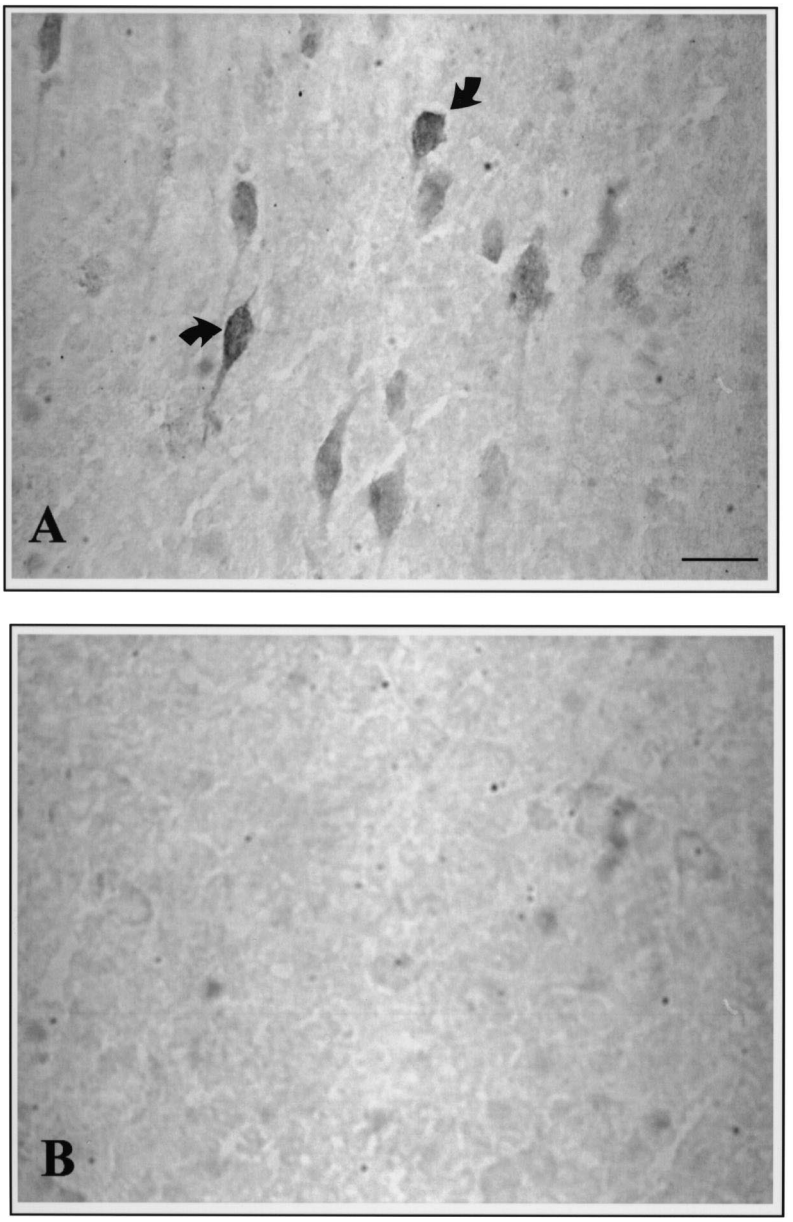

Fig. 2. Immunocytochemistry with anti-IGFBP-3 in non-preincubated (A) and preincubated (B) slices of frontal cortex. IGFBP-3 is located on neuronal cell bodies (arrows). After preincubation for 15 min at 20°C in 25 mM Tris–HCl (pH 7.4), containing 10 mM $MgCl_2$, and 0.1% bovine serum albumin, IGFBP-3 is no longer detectable. Bar=50 μm.

detected in substantia nigra and periaquaductal gray. Low binding was also observed in the red nucleus. In the pons moderate levels of IGF-II receptors were associated with the pontine nuclei. Moderate levels of IGF-II receptors were also observed in the pallidium, amygdala, and thalamus. We found no IGF-II receptors in the white matter. In many brain areas, such as the striatum, cerebral cortex, and hippocampus, the distribution pattern of IGF-II receptors is comparable to that of IGF-I receptors [7]. The most striking difference is that in the cerebellar cortex highest labeling was observed in the granular cell layer, whereas IGF-I receptors predominate in the molecular cell layer [7].

The finding that discrete neuronal regions contain higher levels of IGF-II receptors may suggest a more specialized role of IGF-II as trophic/survival factor or perhaps as neuromodulator for well-defined neuronal systems. The role of the IGF-II receptors in the different neuronal regions is not well understood. In vitro, IGF-II mimics the mitogenic actions of IGF-I, through stimulation of IGF-I receptors [6,15]. In human brain IGF-I has a ten times higher affinity than IGF-II for the IGF-I receptor [7]. On

Table 1
Regional distribution of IGF-II receptors in human brain[a]

Region	Receptor concentration in fmol/mg protein (mean±S.E.M.)
Frontal, parietal, occipital, and temporal cortex	29±7
Cerebral white matter	ND
Corpus callosum	ND
Caudate nucleus, putamen, accumbens	27±5
Pallidium	13±3
Thalamus	10±3
Amygdala	22±3
Claustrum	15±4
Hippocampus	
Pyramidal cell layer	28±2
Dentate gyrus	16±3
Choroid plexus	58±9
Cerebellum	
Molecular layer	13±1
Granular layer	33±6
White matter	ND
Midbrain	
Substantia nigra	21±4
Red nucleus	7±1
Periaquaductal gray	15±4
Pons	
Pontine nuclei	26±3

[a] Data were generated in brain samples from six individuals. ND, not detectable.

the other hand, we found in this study that IGF-II was ten times more potent than IGF-I in displacing [^{125}I]IGF-II. These findings indicate that in human brain, under physiological conditions, IGF-I preferentially interacts with IGF-I receptors, and IGF-II with IGF-II receptors. In vitro observations suggest that IGF-II receptors may function in diverse biologic processes. IGF-II may act as a modulator for the trafficking of mannose-6-phosphate bearing lysosomal enzymes into the cell [11,14,18]. Binding of IGF-II to its receptor may also activate a signaling mechanism through interaction with a GTP-binding protein [5,14], the physiological role of which is not yet understood. Konishi et al. [13] found that IGF-II enhanced choline acetyltransferase activity in a dose-dependent manner in cultures of embryonic mouse septal neurons. Part of this effect was related to a specific interaction with IGF-II receptors, and suggested a role of IGF-II receptors in the differentiation of cholinergic neurons. Further evidence for a role of IGF-II in the cholinergic neurotransmitter system was the observation that IGF-II potentiated the K^+-evoked release of acetylcholine from hippocampal slices [12]. Recently, Kang et al. [10] found that retinoic acid also binds to the IGF-II receptor and suggested that the biological consequence of this interaction appears to be the suppression of cell proliferation and/or the induction of apoptosis. Studies in different neurodegenerative disorders may help to clarify the functional roles of IGF-II receptors in human brain.

Chronic plaques of multiple sclerosis were identified by myelin staining (Fig. 4). The plaques contained a dense atrogliosis as visualized by GFAP immunochemistry (not shown). Similar to normal appearing white matter, we could not detect specific binding of [^{125}I]IGF-II to IGF-II receptors in any of these plaques. IGF-II receptor concentrations in cerebral cortex of MS patients (31±3 fmol/mg protein) were not significantly different from those in controls (29±7 fmol/mg protein; Mann–Whitney test). It has previously been reported that IGF-I receptors are present in white matter and astrogliotic plaques from patients with multiple sclerosis, which is consistent with a well-recognized role of IGF-I in oligodendrocyte survival, myelination, and stimulation of astrocytes [21]. The absence of IGF-II receptors in the astrogliotic plaques indicates that IGF-II receptors are not involved in the mechanisms of astrogliosis.

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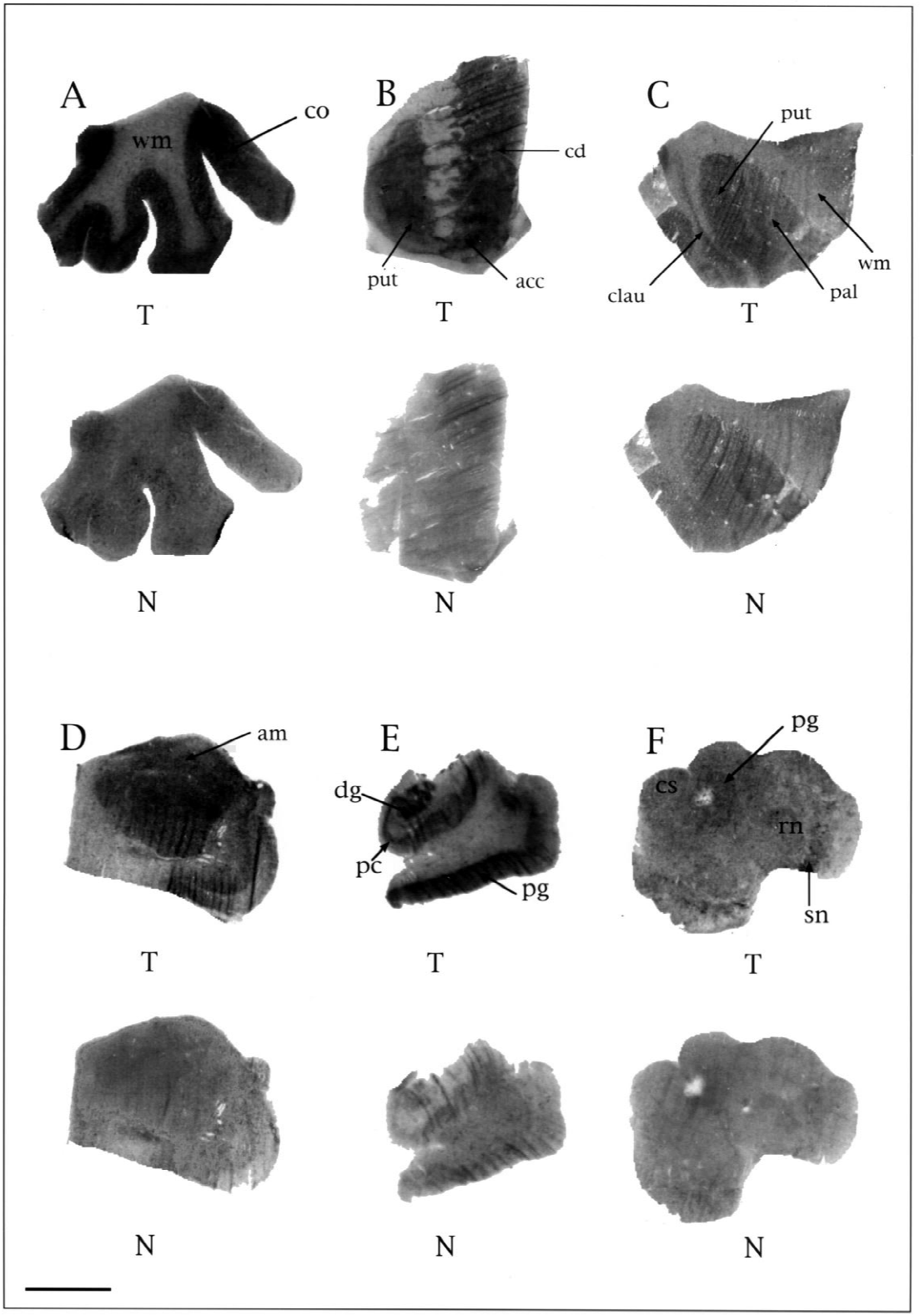

Fig. 3. Autoradiographs of 0.1 nM [^{125}I] IGF-II binding in the absence=total binding (T) and the presence of 0.5 μM unlabeled IGF-II=non-specific binding (N) to microscope slide-mounted sections at the level of (A) frontal cortex (co) and white matter (wm), (B) caudate nucleus (cd)/putamen (put)=neostriatum and nucleus accumbens (acc), (C) claustrum (clau), pallidium (pal) and putamen (put), (D) amygdala (am), (E) hippocampus: dentate gyrus (dg)/pyramidal cell layer (pc), and para-hypocampal gyrus (pg), and (F) midbrain: substantia nigra (sn)/colliculus superior (cs)/red nucleus (rn) and periaquaductal gray (pg). Bar=1 cm.

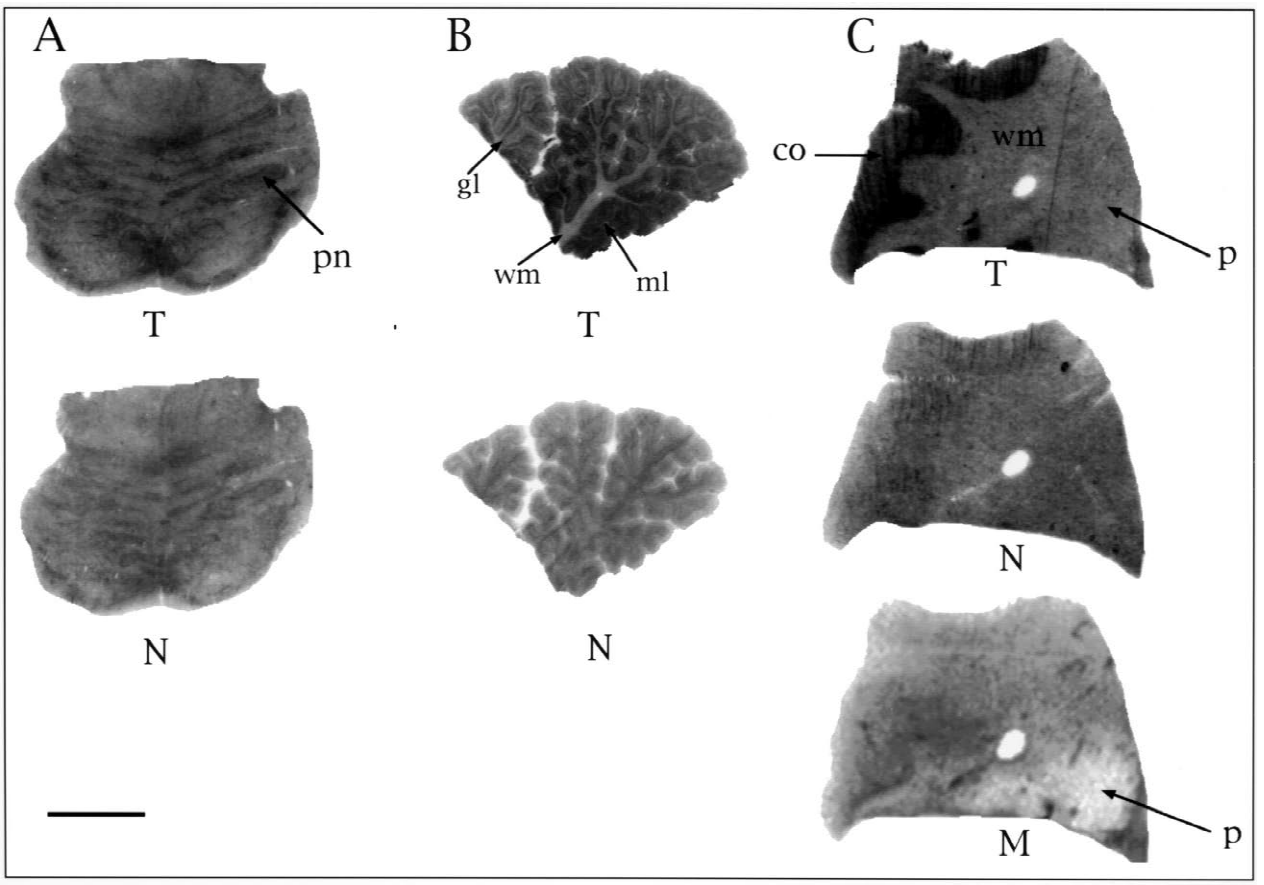

Fig. 4. Autoradiographs of 0.1 nM [^{125}I] IGF-II binding in the absence=total binding (T) and the presence of 0.5 μM unlabeled IGF-II=non-specific binding (N) to microscope slide-mounted sections at the level of (A) pons: pontine nuclei (pn), (B) cerebellum: molecular layer (ml)/granular layer (gl), white matter (wm), and (C) normal appearing white matter (wm), demyelinated astrogliotic plaque (p) and cerebral cortex (co) from a patient with multiple sclerosis. Section (M) shows the myelin staining with luxol fast blue revealing a plaque (p). Bar=1 cm.

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